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Infrared Spectral Imaging of Individual Human Cells at High Spatial Resolution

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Beamline(s): U10B

Introduction: Infrared (IR) spectroscopy has the ability to monitor compositional changes that occur in human cells as a result of cell differentiation, cell division and disease [1-5]. In order to understand these spectral changes, we have embarked in systematic study of cellular spectra under a wide variety of conditions [6-8]. In particular, we are interested in a comparison of the IR spectra observed for the nuclei and the cytoplasm. Prior results have indicated that normal and cancerous cells can be differentiated by their protein : DNA contributions in their spectra [5]. Since DNA is found nearly exclusively in the nucleus of a cell, the differentiation between nuclear and cytoplasmic spectra is of prime importance.

Methods and Materials: *Cell culture:* Benign human skin fibroblasts and malignant bone giant sarcoma cells were purchased from ATCC and grown under standard cell culture protocols. A carefully cleaned, 80 mm x 20 mm x 1 mm CaF_2 substrate was added to the cell culture dish; the cells strongly adhere to such a window and permit easy purification and enzyme digestion procedures to be carried out subsequently. In addition, these windows are ideal for use in IR transmission spectroscopy and microspectroscopy.

Spectroscopic measurements: IR microspectroscopic measurements were carried out using a Nicolet Magna 860 Step-Scan FT-IR instrument coupled to a Spectra Tech Continuum IR microscope at beam line U10B of the vacuum UV ring at the National Synchrotron Light Source (NSLS),. Due to the highly collimated IR-beam of the synchrotron source, apertures as small as 8 x 8 μm^2 could be used with adequate signal quality.

128 interferograms were co-added at 8 cm^{-1} spectral resolution for each pixel. The sample was moved in a raster pattern through the IR beam to collect a spectral hypercube, which was analyzed using software developed in house. This software, implemented in MATLAB (The Mathworks, Natick, MA), was designed for IR imaging and permits data pre-processing as well as uni- and multivariate methods for IR imaging. The software is available for distribution [9].

Results: The spectra observed for benign skin fibroblasts and malignant giant sarcoma cells both are dominated by protein and lipid spectral contribution [10,11]. In fact, the phospholipid bands observed both for nuclei and cytoplasm so dominates the 900 – 1250 cm^{-1} spectral region that DNA and RNA bands could not be identified. Upon treatment of the cells (on the sample substrate) with 100 % ethanol, the lipids could be removed nearly quantitatively, as indicated by the disappearance of the ($C=O_{ester}$) vibration at 1738 cm^{-1}.

After removal of the phospholipids, the observed spectra of both nuclear and cytoplasmic regions were characteristic of protein and nucleic acid contributions. Surprisingly, quite strong nucleic acid signatures were observed in the cytoplasm, presumably from ribosomal RNA. Subsequent treatment of these cells with RNase removed the cytoplasmic RNA nearly completely. The remaining spectral features of the cytoplasm were those of pure protein, whereas the nuclear regions exhibit spectra consistent with protein / DNA contributions. The DNA signatures observed were about the same magnitude for both the sarcoma cells and the fibroblasts.

We have previously reported infrared spectral maps, and a detailed interpretation of the IR spectra, of terminally differentiated and metabolically inactive mucosa cells. In these cells, the nuclei were pyknotic (*i.e.*, shrunk to a very small size). We and others have established that in such cases, the DNA is highly condensed, and does not contribute any IR spectral signatures. Our previous interpretation of this DNA hypochromicity was based on the fact that very small, highly condensed DNA would exhibit an optical density in excess of 10 OD units.

The results presented here have enormously far-reaching consequences for the interpretation and diagnosis of disease by infrared spectroscopic methods. The present efforts have succeeded in answering some of the persistent questions about nucleic acid signals observed during the cell cycle. The spatially resolved data reported here indicate that most of the nucleic acid signals are not due to DNA, but due to RNA, which is found in the cytoplasm of active cells in surprisingly high concentrations. DNA appears to contribute to the observed spectra in actively dividing cells, as postulated from the cell cycle studies.

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